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New HS/FID and traditional enzymatic methods for blood alcohol analysis in Libyan patients with myocardial infarction: A comparative study

Asma O. Jebril [1 *], **Mohammed Al-Rahim** [2], and **Doaa Mansour** [2]

[1] Department of Biochemistry and Clinical Biochemistry, Faculty of Pharmacy, University of Tripoli, Tripoli, Libya
[2] Judicial Expertise and Research Center, Ministry of Justice, Tripoli, Libya
[*] Author to whom correspondence should be addressed

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Abstract: Determination of blood alcohol concentration (BAC) in heart disease patients is crucial for clinical and forensic purposes; enzymatic ethanol assays are widely used to determine blood alcohol content for individuals admitted to a hospital. In some instances, this hospital data is later used as evidence in a court of law. As the enzymatic assay targets, but does not exclusively measure, ethanol, it is theoretically possible that interference could occur and produce a falsely elevated result, leading to wrongful convictions or other consequences. This study examined the potential of clinically relevant levels of one potential interferent, lactate dehydrogenase (LDH) enzyme, to cause significant and falsely elevated results and compared this method with chemical and new HS-GC/FID methods. The study was conducted on 48 individuals (24 myocardial infarction patients, 24 healthy volunteers) in Tripoli, Libya. The BAC (mg/dL) results for healthy and myocardial infarction patients showed a significant difference using the enzymatic method and no difference using the HS-GC/FID method. A clear difference with high significance of BAC appears for myocardial infarction patients by using the enzymatic and HS-GC/FID methods. The Pearson correlation coefficient between LDH levels and BAC by the enzymatic method is 0.76, indicating a strong positive correlation. This suggests that as LDH levels increase, BAC levels also tend to increase (LDH interference may contribute to falsely elevated ethanol readings in myocardial infarction patients), affecting the accuracy of BAC determination. HS-GC/FID is advised over the enzymatic approach, particularly in individuals with myocardial infarction (elevated LDH levels).

Introduction

In clinical diagnostic settings, the assessment of alcohol concentration in blood (BAC) and other biological specimens is a typical request; urgent requests for such tests are often made in emergency clinics and forensic laboratories. Due to its ease of use, rapid, and non-invasiveness, this method is typically chosen when patients can provide breath samples. Additionally, breath analysis measures arterial alcohol levels, which are more physiologically and clinically significant than venous BACs, especially during active absorption stages [1]. For ethanol measurement, clinical and forensic toxicology labs usually use either gas chromatographic or enzymatic methods. Among these, headspace gas chromatography (HS/GC) [2], which has been extensively validated for this particular application, is acknowledged as the reference method in ethanol analysis [3]. Excellent specificity

for ethanol, great analytical sensitivity, and the ability to concurrently detect other alcohols like methanol and isopropanol that may be present with ethanol are just a few benefits of gas chromatography. Although it is currently less popular, one of the first techniques for determining alcohol content was the chemical oxidation approach using potassium dichromate in sulfuric acid solution. This method is quite time-consuming since it requires a lot of sample preparation, including the separation of alcohol from the biological matrix before reacting with the acid-dichromate mixture. Its disadvantages in standard clinical laboratory settings are not sufficiently offset by its reagent stability and simple final measurement. However, since a negative result conclusively rules out the presence of alcohol in the specimen and removes any chemical interferences, the approach may be a helpful confirmatory tool in conjunction with gas chromatographic or ADH procedures [1]. The enzymatic method works on the ethanol oxidation principle, in which nicotinamide adenine dinucleotide (NAD^+) is reduced to its reduced form (NADH) by alcohol dehydrogenase (ADH), **Figure 1**. The concentration of ethanol in the material is directly correlated with the subsequent increase in absorbance at 340 nm [3]. This method's specificity for ethanol is somewhat low, despite its simplicity and quick results. Enzymatic ethanol tests tailored for common clinical laboratory use have been developed by a number of commercial firms [4].

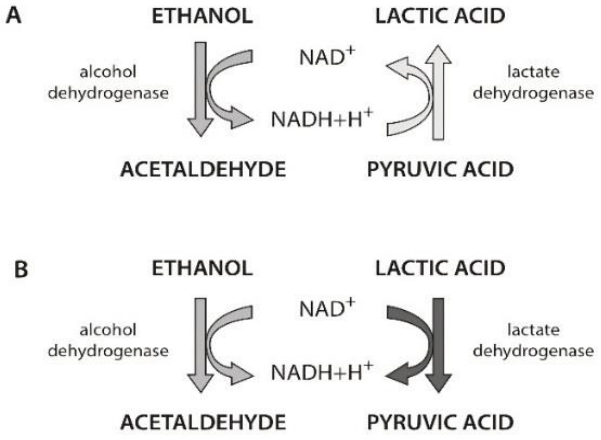

Figure 1: Balance of oxidized and reduced co-enzyme (NAD^+ and NADH + H^+) in the oxidation of ethanol by alcohol dehydrogenase (ADH). In the cytoplasm of hepatocytes. **(a)** Lactate dehydrogenase (LD) consumes pyruvic acid (PA) to restore the NAD^+ equivalents used by ADH to oxidize ethanol to acetaldehyde in samples with elevated concentrations of LD and lactic acid (LA). **(b)** LD reduces LA to PA, producing NADH + H^+, adding to that (if any) produced by ADH used in the assay, leading to a corresponding increase in absorbance at 340 nm.

In emergencies, the ADH-based enzymatic assay is frequently used to quantify serum and BAC levels, enabling the rapid identification of ethanol intoxication. However, some interfering chemicals can affect ADH procedures; lactate dehydrogenase/lactic acid (LD/LA) systems are particularly problematic since they can produce false-positive results. Furthermore, negative bias may be introduced by the presence of free hemoglobin from hemolyzed specimens. The fact that ethanol concentrations detected in plasma or serum are roughly 10.0-20.0% greater than those in whole blood, with a median discrepancy of about 15.0%, must be carefully taken into account when interpreting data from hospital laboratories for legal purposes [5].

There are five different isoenzymes of lactate dehydrogenase (LDH-1 through LDH-5), each with unique subunit compositions and tissue distribution patterns [6]. The LDH-1 isoenzyme is mostly found in cardiac tissue. Elevated levels start to appear 6-12 hours after the onset of chest pain, peak between 1 and 3 days, and then gradually return to normal between 8 and 14 days. Acute myocardial infarction is specifically indicated by an LDH1: LDH2 ratio greater than 1.0 [7]. High LDH1 and LDH2 levels may interfere with enzymatic blood alcohol analysis, leading to erroneously high readings or positive results, according to earlier research [4, 8]. This research expands on initial findings showcased at the 6th EFMA International Conference (2025), offering an extensive comparative evaluation of enzymatic methods against HS-GC/FID techniques for BAC assessment in Libyan myocardial infarction patients.

Materials and methods

Participants: 48 subjects, including healthy (control) and myocardial infarction (MI) patients, aged between 35 and 83 years (59.0 ± 24.0 years). 24 healthy and 24 patients with myocardial infarction (71.0% males, 29.0% females). The samples collected between October 2024 and January 2025 are from the Tripoli Medical Hospital **(**TMH**)**, Mitiga Military Hospital **(**MMH**)**, and National Heart Center **(**NHC**)**, Tripoli, Libya. Healthy volunteers with a history of cardiovascular diseases, cancer, hepatic disease, and kidney diseases were excluded from the study to avoid all conditions that may elevate LDH levels and cause interference. For the control group, the questionnaire included questions about age, sex, and other chronic diseases. Additional questions were added for the patient group questionnaire, including the type of cardiac disease, type of MI (STEMI or NSTEMI), and whether the myocardial infarction occurred within the preceding ten days.

Sample collection: A total of 6.0 mL of whole blood was obtained from each participant and distributed into three distinct tubes. For alcohol analysis procedures, sodium fluoride-potassium oxalate test tubes were utilized and frozen immediately (post-collection) at - 20 °C. Furthermore, clot activator test tubes were employed for the quantification of LDH levels, and this specimen was promptly dispatched to the laboratory (Al-Shark Laboratories for Medical Analysis) following acquisition.

Chemical analysis of blood alcohol: A 1.0 mL blood sample, containing potassium oxalate and sodium fluoride as an anticoagulant, was used. The analysis was conducted through a series of steps that followed the procedure of the Kozelka and Hine/Cavett method [9, 10].

Blood alcohol content by enzymatic method: Followed the procedure by using the ADH enzyme. The enzymatic assay for ethanol and LDH was performed on a BIOLABO Diagnostics (Vietnam) analyzer according to the manufacturer's protocol [11].

Headspace-gas chromatography/flame ionization detection (HS-GC/FID) method: The methodology outlined in this study adhered to Daglioglu and others [8]. HS/GC instrument parameters comprised an initial oven temperature (50 °C), inlet temperature (250 °C), FID temperature (300 °C), HS thermostat temperature (- 92 to 400 °C), HS thermostat times, injection volume (1.0 µL), the flow rate of makeup gas (60 mL/min N_2), and carrier gas (helium 1.0 mL/min^{-1}).

Ethical considerations: The protocol was approved by the Institutional Ethics Committees of the participating hospitals in 10/2024. All the participants were informed about the study, and written consent from each volunteer was obtained prior to the blood sample being taken. A written questionnaire was given to each volunteer. Participant confidentiality and privacy were strictly maintained by anonymizing all collected data (024-MJ-03-MJMR-2026).

Statistical analysis: All statistical analyses were done using the IBM SPSS Version 21 program. The median of the data was calculated using descriptive analysis. Comparative analysis between groups was done using the Mann-Whitney U test. Differences are considered statistically significant at $p < 0.05$.

Results

The BAC (mg/dl) and PAC (mg/dl) for 24 healthy and 24 MI patients were determined using enzymatic and headspace gas chromatography. LDH blood level for all volunteers was measured (U/L).

Lactic dehydrogenase level (LDH): The blood level of LDH in healthy people and patients under study was between 154 and 1399 U/L, as shown in **Table 1**. Healthy volunteers (males and females aged 35 - 75 years)

showed an LDH level in their blood samples between 154 and 220 U/L, which is within the normal range. Patients with myocardial infarction (males and females aged 36 - 83 years) gave LDH levels in their blood samples between 230 and 1399 U/L; all MI patients had a rise in LDH in their blood. Typically, cardiac injury and myocardial infarction samples have high LDH levels because when cardiac tissue is injured during a myocardial infarction, lactate dehydrogenase (LDH) is released into the circulation. In **Table 1**, there is wide variation in LDH blood levels between healthy people and patients, statistically with high significance ($p < 0.05$, $p = 0.0001$).

Table 1: Analysis of LDH blood levels of myocardial infarction patients

LDH levels in U/L				
Group	**Median**	**N**	**Standard deviation**	**P < 0.05**
Healthy Subjects (control)	192.1	24	22.7	
MI patients	632.5	24	349.8	
P = 0.0001				

Evaluated blood alcohol concentration: healthy and myocardial infarction volunteers by chemical method: The method used for detecting alcohol in blood through visual observation. The results were determined as illustrated in **Figure 2.** In the tube labeled "Test" in **Figure 2,** a drop (10.0 µl) of ethanol was added to make the color change to blue from yellow (dichromate color), while the other tubes for MI blood samples showed NO color change detection. Enzymatic method: The enzymatic method is widely used in forensic laboratories due to its low cost, ease of operation, and high throughput. However, it may be susceptible to interference. Blood alcohol concentration (BAC) levels are measured using an enzymatic method.

Figure 2: The findings of the chemical method

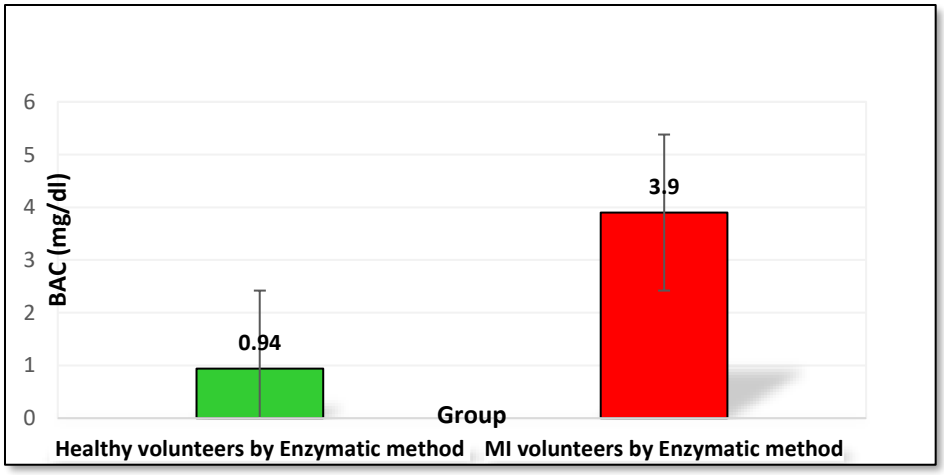

Figure 3: Median BAC by enzymatic method

Table 2: Difference between myocardial infarction patients and healthy individuals

Myocardial infarction volunteers						Healthy volunteers			
Age	**Gender**	LDH (U/L)#	BAC (mg/dl) by Enzymatic method	PAC (mg/dl) by GC-HS/FID method	PAC (mg/dl) by GC-HS/FID method	BAC (mg/dl) by enzymatic method	LDH (U/L)#	**Gender**	**Age**
36	M	544	3.75	-	-	1.08	203	M	35
47	M	857	15.77	-	-	0.83	187	F	40
55	M	729	7.25	-	-	0.14	180	M	44
55	M	652	4.06	-	-	2.07	176	F	45
56	M	857	5.49	-	-	1.48	193.3	M	52
56	F	1399	12.18	-	-	0.14	147	M	52
57	M	865	3.058	-	-	1.76	154	M	53
58	F	613	4.06	-	-	1.14	220	M	53
58	M	1238.8	17.25	-	-	1.05	215	F	53
59	M	891	3.1	-	-	0.47	198	M	54
59	F	1296	16.34	-	-	0.19	163	F	55
63	M	329	0.81	-	-	0.57	163	M	56
65	M	550	16.34	-	-	0.66	191	F	57
66	F	230	2.34	-	-	1.19	156	M	58
67	M	380	2.62	-	-	0.47	200	M	60
68	M	470	3.73	-	-	0.43	218	M	61
68	M	265	2.48	-	-	1.19	203	M	65
69	M	657	3.15	-	-	0.19	195	M	65
71	M	511	2.62	-	-	0.66	220	F	68
73	M	876	10.22	-	-	1.81	191	F	69
73	F	460	4.87	-	-	1.09	172	M	69
75	F	1359	18.33	-	-	0.09	160	F	71
76	F	311	1.05	-	-	1.29	208	M	74
83	M	390.8	2.34	-	-	1.48	215	M	75

In MI patients, BAC ranges between 0.81 and 18.33 mg/dl, whereas in healthy subjects, the range is from 0.09 to 2.07 mg/dl. The median BAC in MI patients is significantly higher (3.91 mg/dl) than the median in healthy. **Table 2:** The main table result of MI and healthy volunteers by enzymatic and HS-GC/FID methods. The normal range for the LDH enzyme in blood is 135-225 IU/L. (0.94 mg/dl) individuals **(Figure 3)**. These findings suggest that MI patients generally have higher BAC levels compared with healthy individuals.

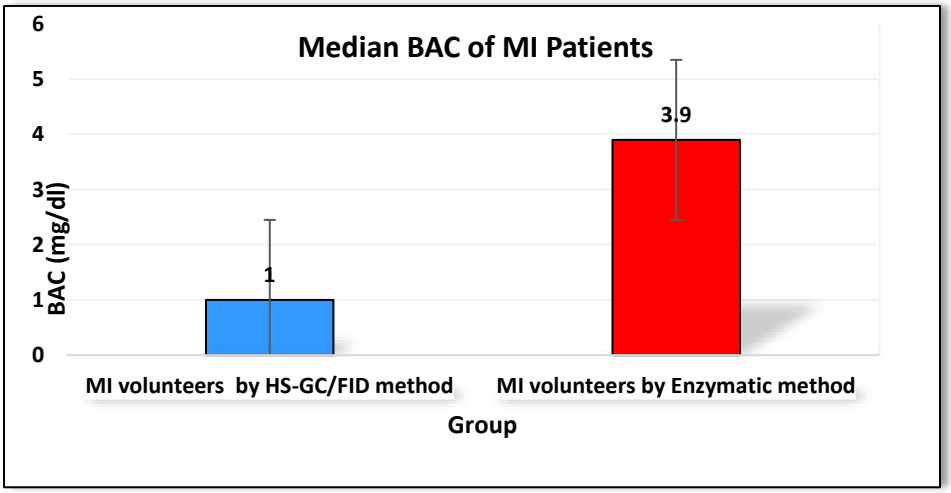

Figure 3: Column chart of median BAC by Enzymatic method

The difference in median BAC levels between myocardial infarction (MI) patients and healthy volunteers can be attributed to metabolic changes in MI patients. MI causes tissue damage, leading to increased anaerobic metabolism and elevated lactate and lactate dehydrogenase (LDH) levels. This metabolic shift can interfere with enzymatic assays. Resulting in falsely elevated BAC readings. Healthy volunteers, lacking these metabolic alterations, show a much lower median value (MD=0.9). HS-GC/FID method: The HS-GC/FID technique offers elevated sensitivity and specificity for organic substances. The findings indicated that the ethanol concentration was below 0.0001 mg/dL in both healthy individuals and patients with myocardial infarction whose samples were collected without antiseptic alcohol for skin preparation.

The higher median BAC value of 3.9 mg/dL (**Figure 4)** observed in MI patients using the enzymatic method suggests possible interference, likely from lactate dehydrogenase (LDH). Evaluating the BAC (mg/dl) outcomes by enzymatic technique and HS-GC/FID method for myocardial infarction patients: **Figure 4** shows a high significance in BAC (mg/dl) between MI patients using both enzymatic (MD=3.9) and HS-GC/FID (MD = 1) methods, with statistical significance ($p < 0.05$, $p = 0.0001$) in **Table 3**.

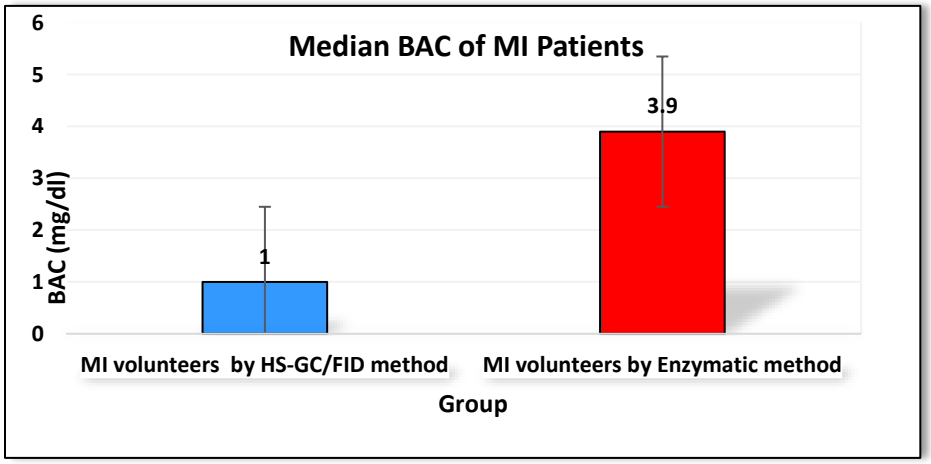

Figure 4: Median BAC through enzymatic and HS-GC/FID methodologies

Table 3: Analysis of myocardial infarction patients by using enzymatic and HS-GC/FID methods

MI Patients				P < 0.05
Group	**Median**	**N**	**Standard deviation**	
BAC (mg/dl) by enzymatic method	3.9	24	5.8	
PAC (mg/dl) by HS-GC/FID method	1	24	1.31	
P = 0.0001				

Discussion

This significant difference indicates that BAC results obtained using the enzymatic method in MI patients may not be reliable. Such discrepancies have important forensic and legal consequences, as false-positive alcohol levels could lead to wrongful accusations or misinterpretations in both medical and legal settings. The potential for inaccurate BAC readings in MI patients using enzymatic methods underscores the need for caution when interpreting these results, especially in contexts where the implications can be serious. It highlights the importance of using more specific and accurate methods, such as HS-GC/FID, particularly when dealing with patients who have experienced myocardial infarction or in situations where precise BAC measurements are crucial for legal or medical decision-making [12]. MI is a severe cardiovascular event characterized by the death of heart muscle

tissue due to insufficient blood supply [13, 14]. One of the key biochemical markers used in MI diagnosis is lactate dehydrogenase (LDH), specifically LDH-1, which is predominantly found in cardiac tissue [8]. Following an MI, LDH levels rise within 6-12 hours, peak between 24 and 48 hours, and can remain elevated for up to 10-14 days, making it useful for late-stage diagnosis when other markers, such as troponins, start to decline. However, LDH is a valuable marker but lacks cardiac specificity because it is present in various tissues, including liver, kidneys, and skeletal muscles [15]. This limits its standalone diagnostic accuracy for MI.

In the present study, in STEMI, LDH elevation tends to be more pronounced due to the extent of myocardial damage in six patients with MI, whereas in NSTEMI, the rise in LDH may be less significant but still detectable in 11 patients [16]. The enzymatic method is widely used in forensic laboratories due to its low cost, ease of operation, and high throughput. However, it may be susceptible to interference. This study corroborates that interference in ADH enzymatic assays due to LDH enzyme in MI patients, while infrequent, can be noteworthy if overlooked. False positive results are possible, but only when LDH concentrations significantly exceed typical physiological levels (when they exceed 800 IU/L; normal LDH level is 135-225 IU/L). The potential for such interference, even if rare, raises concerns about possible misinterpretation or misuse of otherwise valid assay results in medical or legal settings. This highlights the critical need to recognize and comprehend the specific conditions that may lead to this interference, ensuring accurate interpretation of ADH enzymatic assay outcomes. By understanding these factors.

Clinical laboratory workers in the chemistry and toxicology laboratories at the Center for Judicial Expertise and Research, using enzymatic ethanol assays, should be aware of potential causes of false-positive or falsely elevated ethanol results. Laboratory policies or result flags may be instituted to alert clinicians to order a second ethanol analysis by a definitive Analytical method such as HS-GC/FID or to perform a second enzymatic alcohol measurement on the same specimen collected 1 hour or more later, thus allowing for the metabolism of any true ethanol [17, 18]. HS-GC/FID, on the other hand, is considered more specific, especially at concentrations near legal limits. It also allows for the detection of other alcohols that may co-occur with ethanol. In Libya, the consumption of alcohol is strictly prohibited by law, and any detectable level of alcohol in the blood is considered illegal. In the centers of judicial expertise and research, the concentration is 0.01% (10 mg/dl) as the beginning of the signs of drunkenness in the accused. The findings of this study are crucial for forensic toxicologists in interpreting hospital enzymatic alcohol assay results.

Conclusion: Enzymatic assays systematically overestimate BAC in myocardial infarction patients due to endogenous LDH interference, whereas HS-GC/FID provides a clean, accurate measurement. Hospital laboratories, particularly within Libya where forensic verification may be required, should adopt HS-GC/FID for patients presenting with acute cardiac markers. Relying exclusively on enzymatic testing risks misinterpreting a standard physiological response to cardiac injury as alcohol intoxication.

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Author contribution: MA conceived and designed the study. DAM collected the data. AOJ & DAM performed data analysis and drafted the manuscript. All authors approved the final version of the manuscript and agreed to be accountable for its contents.
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Data availability statement: The raw data that support the findings of this article are available from the corresponding author upon reasonable request.
Author declarations: The authors confirm that they have followed all relevant ethical guidelines and obtained any necessary IRB and/or ethics committee approvals.
Generative AI disclosure: No Generative AI was used in the preparation of this manuscript.